

# *Gradient Device for the Study of Temperature Effects on Biological Systems*<sup>1</sup>

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## **Introduction**

### *Need for Closely Spaced Measurements*

Over a period of about two decades in the early part of this century a fundamental change in attitude occurred regarding experimental work in the natural sciences. Profound insight was being made possible with the advance of atomic and molecular theories. The scope of possible research was expanded almost beyond imagination. Vast new areas of science became accessible and to advance over such a broad front dictated a philosophy of experimentation which did not permit each phenomenon to be subject to intense, detailed study. The "romance of the next digit" faded. As an example, in studies of thermal properties of matter it became possible at this time to extend measurements from very close to the absolute zero to very high temperatures and few researchers saw the need to measure, for instance, surface tension of water at one degree intervals. This attitude persisted and by the middle of the century the introduction of computers dealt the final blow to "closely spaced measurements": it seemed more appropriate to make measurements with the highest possible precision at a smaller number of points and let the computer provide best fit to the data for interpolation- and extrapolation-purposes.

Unfortunately, it is not always realized that failure to make measurements at closely spaced intervals of the independent variable tacitly implies that the dependent vari-

able is a relatively slowly varying, continuous, monotone function of the independent variable. This (unstated) assumption is frequently fully justified. However, it remains nonetheless an assumption and at least in studies where water at interfaces (vicinal water) is involved, the assumption of a simple dependency on temperature of the parameters observed is not obeyed.

In this paper is described a device—based on maintaining a thermal gradient in a metal block—which facilitates making measurements at closely spaced temperature intervals. In addition, some of the types of results obtained with a "gradient incubator" in our laboratory will be discussed. The studies range from measurements of physicochemical properties of relatively well-defined, simple systems to the study of complex biochemical and physiological processes.

### *A Classical Example: Viscosity*

In 1936, Magat<sup>1</sup> proposed that unexpected, relatively abrupt changes occur in the properties of water (and aqueous solutions) around 40 to 50°C. Among the properties reported to be "unusual" was the viscosity (of pure water) and also surface tension.<sup>2</sup> On this basis, Magat inferred that a change occurs in the structure of water in this temperature range. A number of authors expanded on this notion, including the late J. D. Bernal in whose laboratories measurements of the viscosity of pure water were carried out at closely spaced temperature intervals. Many papers, some quite controversial, were published on this

<sup>1</sup> Contribution #28 LWR

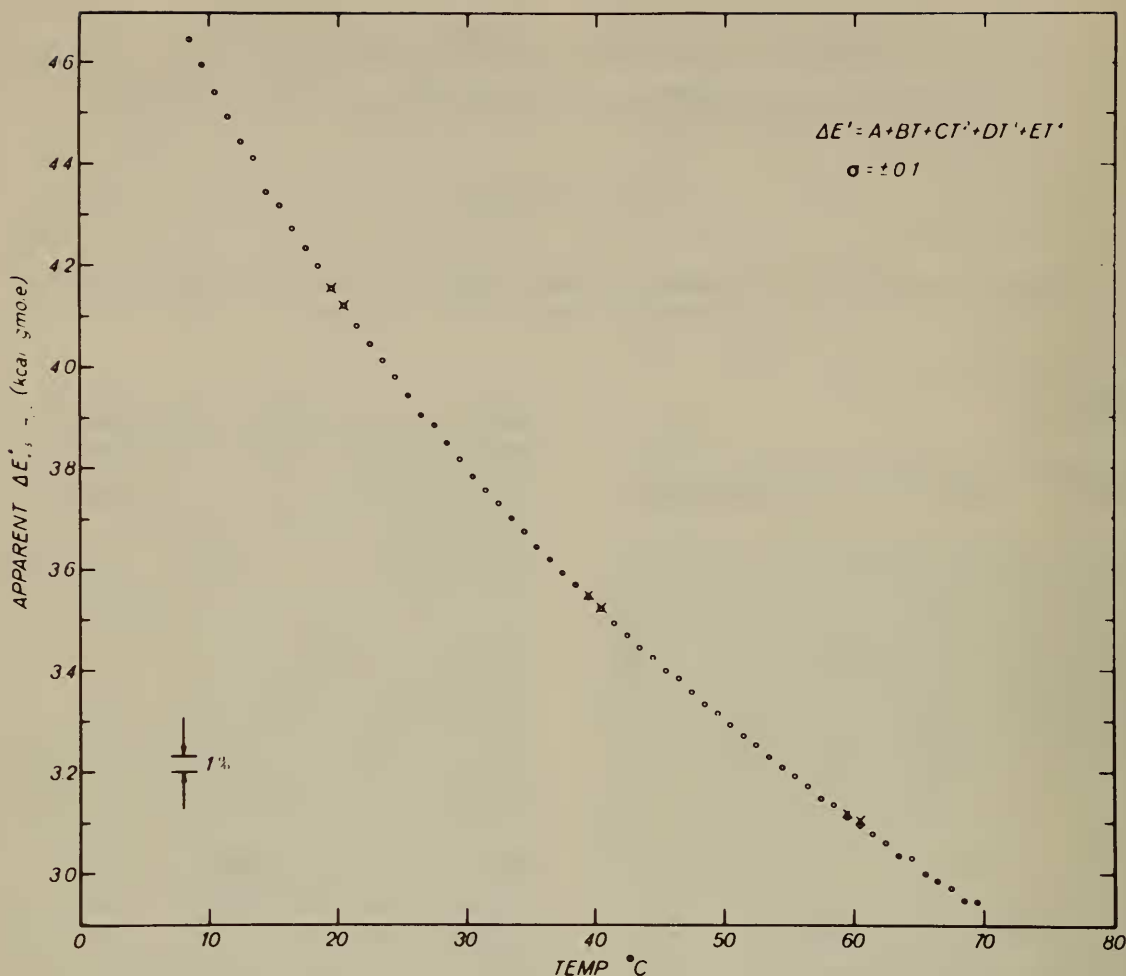


Fig. 1. Energy of activation for viscous flow of bulk water. (Korson, Millero, Drost-Hansen; 6)

subject and reviews of the state of affairs by the mid sixties were presented by the present author.<sup>3-5</sup> Because of the highly controversial nature of the phenomenon we decided to carry out detailed measurements of the viscosity of water using the highest precision attainable at closely spaced intervals. The results<sup>6</sup> clearly demonstrated that no unusual temperature effects existed in the viscosity data for bulk water. Yet enough reliable measurements had been reported by a large number of authors of anomalous properties of water to warrant a detailed search for the origin of these anomalies. The result of this effort was the suggestion<sup>7</sup> that bulk water does *not* exhibit any anomalous changes with temperature but that the properties of *vicinal water* (i.e., water at interfaces, particularly near solid surfaces) indeed *undergoes abrupt changes as a function of temperature* at various temperature intervals. In many of the sys-

tems studied, the effects of vicinal water were superimposed on the primary (bulk) quantity being measured, thus accounting for persistent but variable and poorly reproducible anomalous effects. In the case of viscosity measurements this was demonstrated particularly well by the results obtained by Peschel and Adlfinger.<sup>8</sup> Figure 1 shows the entirely "normal" temperature dependence of the apparent energy of activation for bulk water,<sup>6</sup> while Figure 2 shows the unusual viscosity of vicinal water as reported by Peschel and Adlfinger. Note that closely spaced measurements were made in both our own measurements and in those of Peschel and Adlfinger.

In connection with closely spaced measurements of viscosity, it is also of interest to note the study by Plötze, *et al.*<sup>9</sup> on the viscosity of kaolin suspensions. Their results are shown in Figure 3. Again, an anomaly is observed—in this case as a

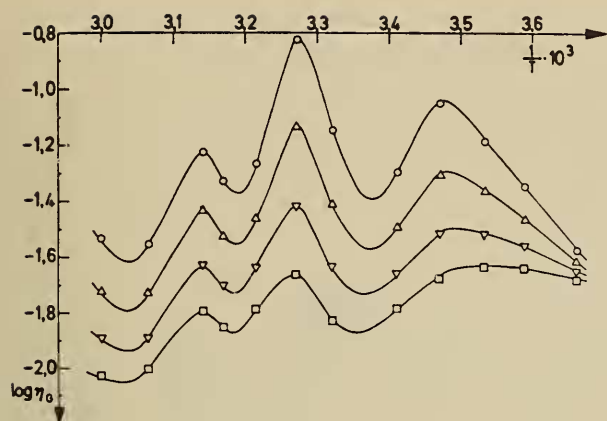


Fig. 2. Arrhenius plot of viscosity of water between two quartz plates. Distance between plates: (bottom to top) 900, 700, 500 and 300 Å. (Peschel and Adlfinger, 8)

function of concentration. Whether or not the anomaly reflects effects of vicinal water (such as overlap of "hydration hulls" of the clay platelets) is not of primary importance here. Suffice it to point out that this anomaly is so "sharp" that it was observed only by virtue of the very closely spaced measurements carried out.

## Thermal Gradient Devices

### Historical Note

One of the first uses of a thermal gradient device appears to have been reported by Herter (see reference 10, pages 148–149). His gradient block consisted of a massive metal bar, heated in one end (by a Bunsen burner) and cooled at the other end, apparently with no automatic control device but relying on manual regulation. While the instrument was lacking in sophistication, Herter nonetheless deserves credit for the first use of such a gradient approach in ecological laboratory studies (thermotaxis of insects).

The use of gradient devices—especially for the study of ecological effects of chemicals on aquatic organisms—goes back more than 60 years, to Shelford and Allee.<sup>11</sup> A review of early work can be found in a paper by Höglund<sup>12</sup> from 1951 who introduced an improved design of a chemical gradient device, referred to as a "fluvium".

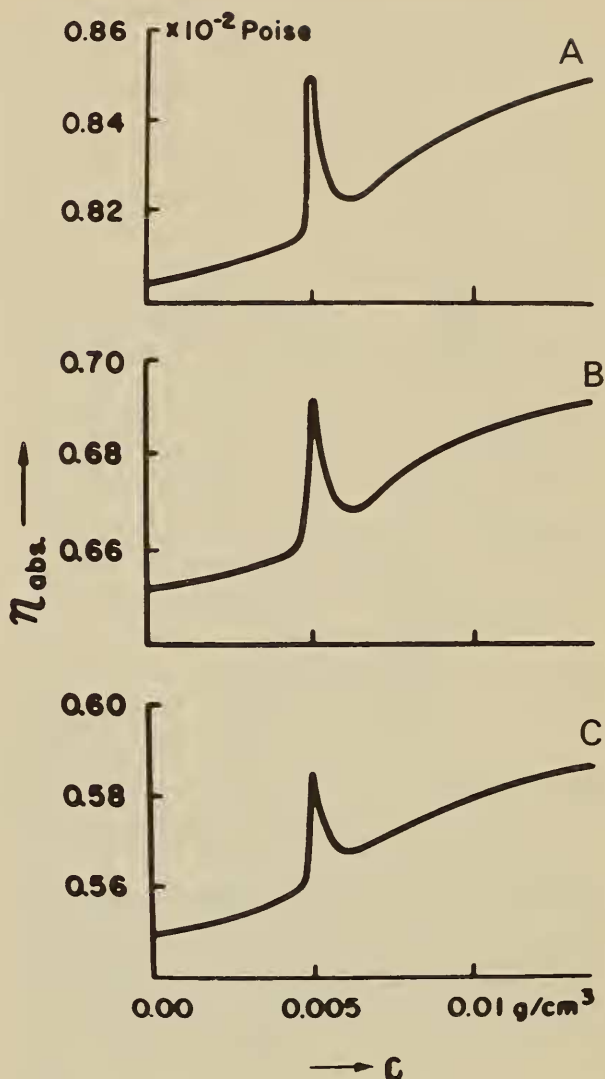


Fig. 3. Apparent viscosity of dilute kaolin suspensions, at three different temperatures: A) 30°C; B) 40°C; C) 50°C. (Plötze *et al.*, 9)

In the same decade, at least three thermal gradient devices were constructed. Scott and Jones at Scripps Institution of Oceanography (in California) in 1958 described a temperature gradient incubator in a relatively inaccessible ONR report.<sup>13</sup> Halldal and French,<sup>14</sup> also in 1958, described an ingenious "cross gradient culture chamber" in which (continuous) gradients of temperature and light intensity were maintained. The device was used for the study of growth of algae. Finally, Oppenheimer and Drost-Hansen<sup>15</sup>—somewhat earlier but unaware at the time of the previous work by Herter—constructed a "polythermostat", originally intended primarily for bacterial growth experiments. It is this device which will be

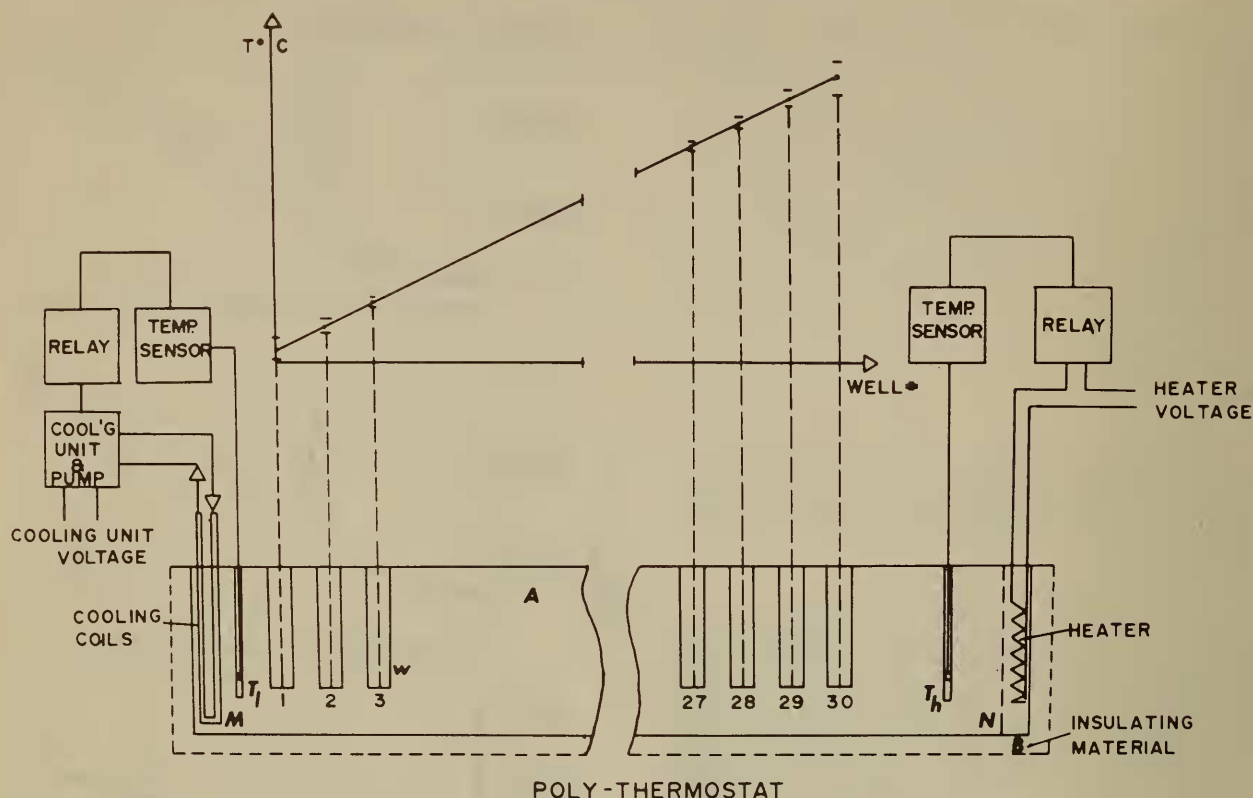


Fig. 4. Polythermostat (Temperature Gradient Incubator); schematic. See text for details.

discussed in the present paper. However, attention is called as well to a number of papers by other authors in which polythermostats have also been described.<sup>16</sup>

### Instrument Design

Oppenheimer and Drost-Hansen first constructed the thermal gradient device for bacteriological studies in 1956.<sup>15</sup> The principle used is simple. In general, temperature gradient bars (and gradient plates) depend on the high heat conductivity of a suitable metal block, insulated on the sides and maintained at fixed constant temperatures at opposite ends (or sides). Figure 4 shows schematically the principle usually employed in the construction of temperature gradient bars. *A* is the heat conducting metal block, usually aluminum (the heat conductivity of aluminum being very high). The gradient bar is provided with wells (usually two rows arranged symmetrically) to accommodate culture tubes or test tubes. *C* and *D* are, respectively, a heating element and a heat exchanger, such as cooling coils. The entire assembly is covered on all sides

(except possibly for the top, or "working side") with suitable insulation material such as polystyrene foam (or other materials; for instance, Bakelite, for working at higher temperatures).

The temperatures at each end are maintained constant by the use of two temperature controllers such as mercury on/off switches, platinum resistance sensors, thermistors or (in earlier versions) bi-metallic contacts. The cooling is usually provided by circulating a cold liquid either from an external constant-temperature cooling unit or from a built-in refrigeration unit (in some commercial models). For certain operations where very low temperatures are not required, cold tap water may suffice to maintain a fixed (low) temperature.

Temperature linearity and fluctuations in the gradient bar are determined by the sensitivity of the thermoregulators at the hot and cold ends and by any spurious heat transfer to or from the surroundings (due to imperfect insulation). In our own experience the gradient is usually quite linear; however, for other reasons (to be discussed below), the actual gradient is normally de-

terminated from a calibration run prior to (or simultaneously with) actual experimentation.

In many applications, particularly those involving sample containers other than test tubes or culture tubes (which are completely contained within the sample wells) it is necessary to perform a calibration. In our own experiments we have usually also maintained two or three wells without samples to spot-check for temperature constancy during individual experiments. Using L-shaped tubes or Thunberg tubes a significant amount of the tube extends beyond the thermally conducting, temperature regulated, part of the aluminum bar. In these cases, a measurement of the actual temperature of the tube is necessary as the final temperature is somewhat different from the calculated temperature (due to the gradient itself) as it is influenced by the ambient temperature (say, within  $\pm 1$  to  $3^{\circ}\text{C}$ ); the constancy of the tubes is satisfactory.

The overall temperature constancy is determined primarily by the temperature regulation at the ends of the bar. The temperature fluctuations depend critically on the relative locations of the temperature sensors with respect to the heaters and cooling coils. The tendency is for the temperatures at the extremes—in Figure 4, well number 1 and to a lesser extent number 2, and well number 30 (and number 29)—to vary within some tenths (to one) degree. For the majority of the wells (say, numbers 3–28), the temperature variations are usually within 0.2 degrees, or better. For special applications, greater temperature constancies may be required. In those cases it is necessary to use highly sensitive thermo-regulators (such as mercury-in-glass metastatic on/off controllers, sensitive to  $0.005^{\circ}\text{C}$  or better). It is possible to bring the temperature variations of the major part of the bar to  $\pm 0.02$  (to  $0.05^{\circ}\text{C}$ ). For most purposes this appears to be sufficient.

### *Sample Containers*

A variety of sample containers have been described in the literature and various

types have been developed in our laboratory.

Ordinary test tubes, usually stoppered with cork or rubber stoppers, have frequently been employed and have proven highly versatile. In work with bacterial cultures, standard screw cap culture tubes have been used. Available commercially are also various types of “L-shaped” tubes. Some slightly curved “L-shaped” tubes have been used in the author’s laboratory, but are primarily useful only for specialty applications. The use of culture flasks in thermal gradient devices have been described by Miller and co-workers.

Commercial temperature gradient incubators are available with provisions for culture trays (made of stainless steel) or long glass tubes which can be filled with the inoculated medium. In principle, such tubes may be monitored either visually or by passing through a spectrophotometer with a suitably modified sample holder design. The use of sample trays is particularly employed in temperature gradient plates; for instance, for determination of seed germination or growth plants.

For bacterial growth experiments as well as for some enzyme reactions, the classical Thunberg tubes have been employed in our laboratory. Using such tubes it is obviously possible to study the growth of anaerobic organisms. However, one experimental difficulty is frequently encountered; namely evaporation of the medium and condensation of the liquid in the part of the tube not contained within the temperature controlled portion of the gradient bar.

### *Agitation of Samples*

In the original version of the temperature gradient device, constructed by Oppenheimer and Drost-Hansen, the samples were contained in vertical wells in the bar. To agitate the contents of the tubes the entire unit was mounted on a sieve-shaker. While this approach offered some agitation in the tubes, it was noted that in some cases standing waves resulted with “nodes” with no or little agitation compared to the tubes in other wells. (A com-

mercial model was available some years ago with a built-in eccentric motor also serving to shake the entire bar with its auxiliary instrumentation).

In two currently available models of the gradient device the samples are contained in horizontal wells in the gradient bar. In this case the same samples can be left at rest or tilted at a certain angle. The samples can also be tilted with a variable period around a horizontal axis through the length of the aluminum bar. In this case effective agitation is obtained; the amplitude and frequency of the rocking motion may be adjusted by changing the point of attachment of the bar to an eccentric wheel-and-cam arrangement.

### Temperature Ranges

One of the commercially available models (manufactured by Scientific Industries, Inc., of Bohemia, New York) is designed for use over a relatively wide temperature range. Thus, the temperature of the cold end may be selected in the range between  $-5^{\circ}$  and about  $20^{\circ}$ , while the temperature at the hot end may be set between approximately room temperature and  $105^{\circ}\text{C}$ . The model employs a total of two horizontal rows of 30 wells each. The access to the two sets of 30 samples are from opposite sides of the gradient bar. At operations (of the low end) below the dew-point in the laboratory, ice tends to form on the exposed filling part for the circulating coolant liquid; however, this does not seem to affect the operation of the instrument significantly. The instrument is highly versatile and one such unit has been operating nearly continuously in the author's laboratory for three years.

## Applications

### Physico-chemical Systems

#### Sedimentation Studies

Using a polythermostat in which the samples are contained in vertical test tubes,

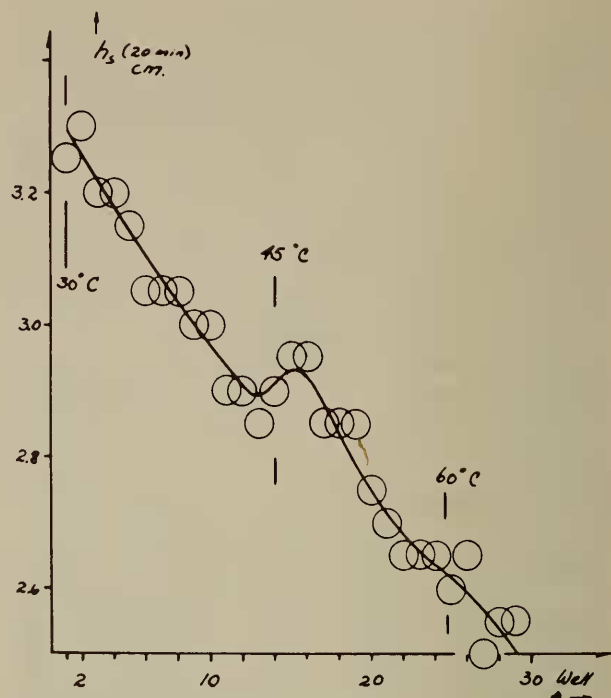


Fig. 5. Sediment height in 10% kaolin suspension (after 20 min.) as function of temperature. (Drost-Hansen, 17)

rates of sedimentation and compaction of dispersed solids have been measured. So far measurements have been made primarily on kaolin and on suspensions of polystyrene spheres (of uniform diameter, produced by Dow Chemical Co.).

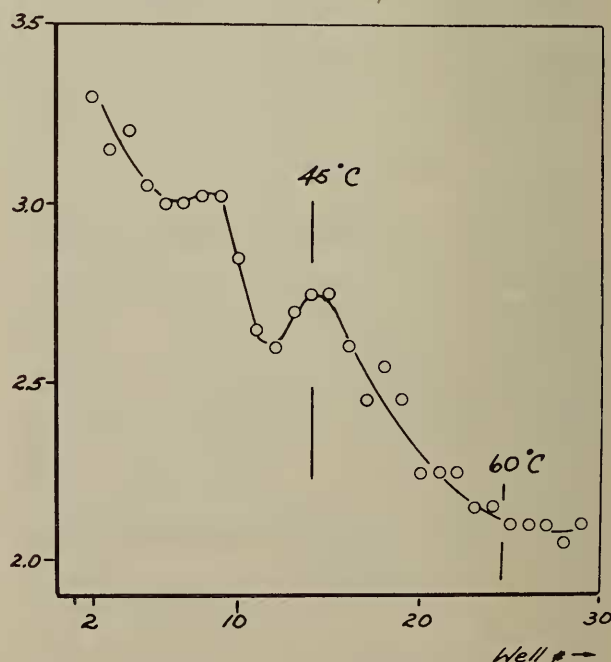


Fig. 6. Sediment height in 10% kaolin suspension (after 7 hours) as function of temperature. (Drost-Hansen, 17)

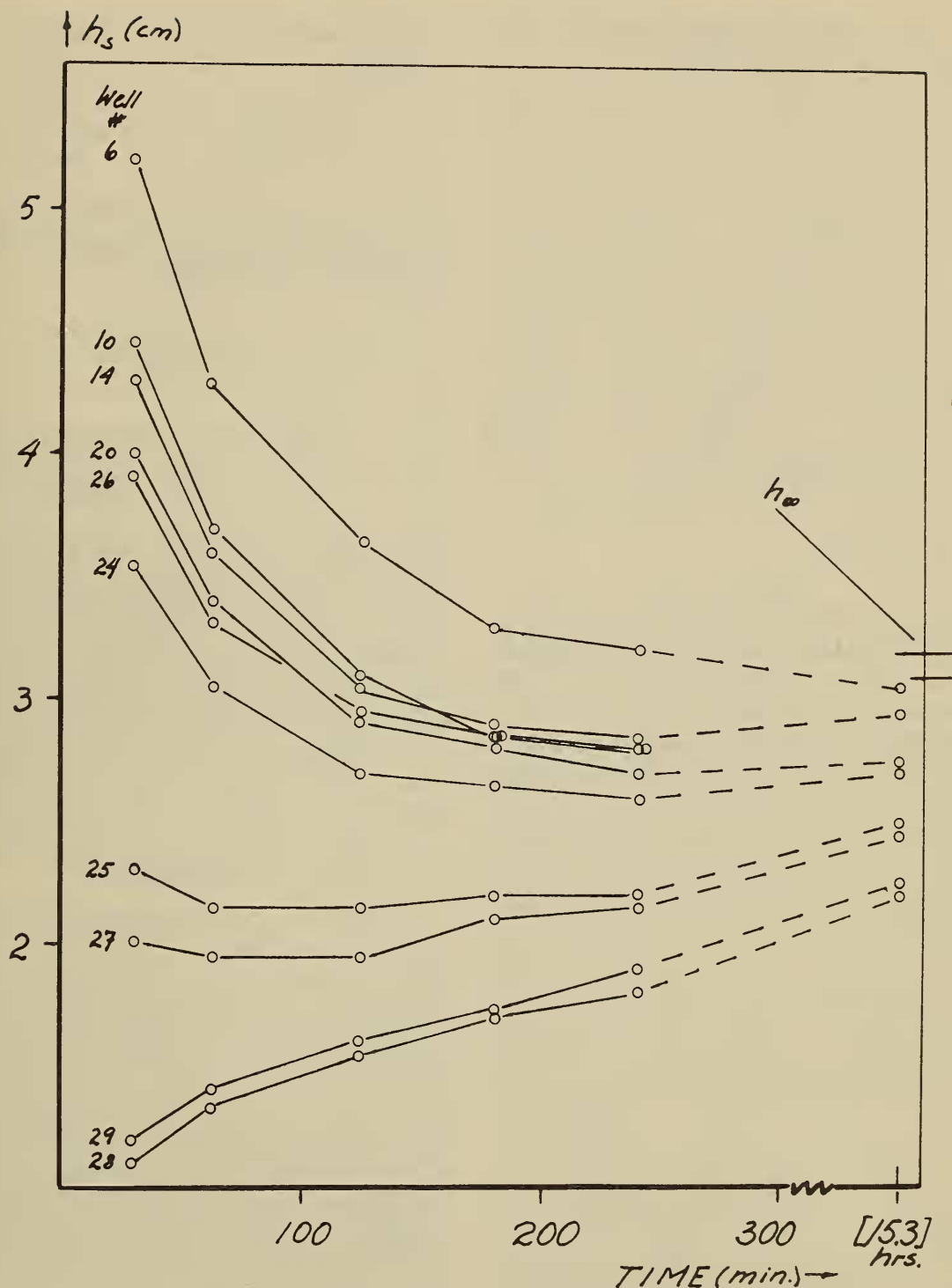


Fig. 7. "Sedimentation isotherms" of 8% kaolin as function of time for various temperatures. (approx. temperatures, for well #6; 10; 14; 20, and 25 are, respectively, 36; 40.5; 45; 53 and 60°C)

The study of the sedimentation of kaolin has been made by simply measuring the height of the sediment in each test tube with a ruler. Two typical sets of data are shown in Figures 5 and 6. In the first of these illustrations is shown the sediment height 20 minutes after the samples had been thoroughly agitated; in the second illustration,

after seven hours of sedimentation. Note in Figure 5 (a) the decrease in sediment volume with increasing temperature, and (b) the anomaly around 45°C (confer Figure 2 in which an anomaly exists in the viscosity of vicinal water at this temperature). For comments on these data and implications of vicinal water for colloidal stability, see

(17). The process of sedimentation and compaction of natural kaolin appear to be more complex than originally expected. Figure 7 shows some "sedimentation isotherms" (all conveniently obtained simultaneously in a single run). The data are somewhat unexpected, no doubt due in part to notably different rates of actual sedimentation and subsequent compaction. An analysis of the data in terms of the individual contributions is not possible without additional information, but the results of the experiment suggest that further study may be highly worthwhile and such work is in progress in our laboratory.

### *Ion Distribution*

In 1975 Wiggins<sup>18</sup> in New Zealand reported on measurements of the distribution of sodium and potassium ions from equimolar sodium-potassium salt solutions in contact with a highly porous quartz gel. Wiggins' results are shown in Figure 8. The

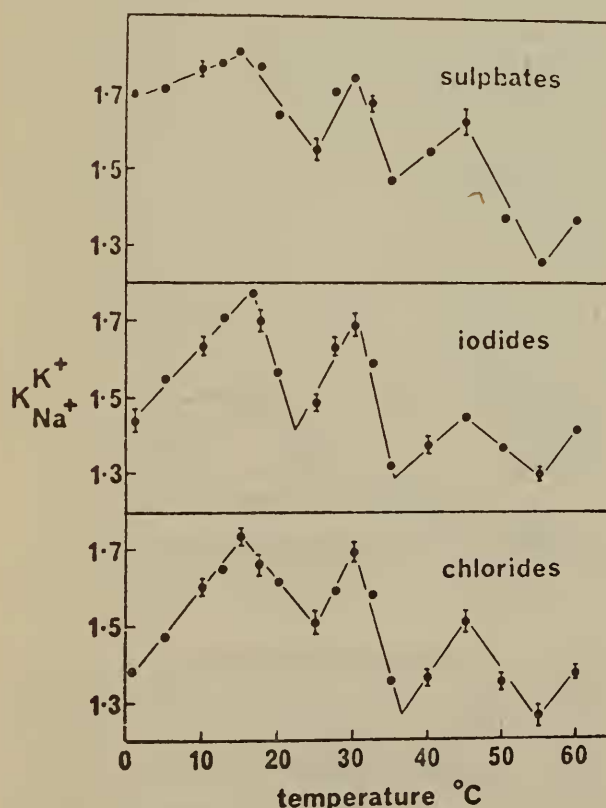


Fig. 8. Potassium/sodium ion distribution in silica pores (from equimolar mixture of  $\text{Na}^+$  and  $\text{K}^+$ ) as function of temperature. Top to bottom: sulfates; iodides; chlorides. (Wiggins, 18)

partition coefficient,  $K$ , is defined by the equations:

$$\lambda_{\text{K}^+} = \frac{[\text{K}^+]_i}{[\text{K}^+]_o} \quad \lambda_{\text{Na}^+} = \frac{[\text{Na}^+]_i}{[\text{Na}^+]_o}$$

where  $[\ ]_i$  and  $[\ ]_o$  are, respectively, the ion concentrations inside and outside the pores of the gel. Hence,

$$K = \frac{\lambda_{\text{K}^+}}{\lambda_{\text{Na}^+}}$$

The values for  $K$  were calculated for potassium and sodium sulfate, iodide, and chloride. As can be seen from Figure 8, potassium ions tend to exclude sodium ions, the value of  $K$  ranging from 1.3 to 1.7. The distribution is seen to be highly nonlinear with sharp peaks near 15, 30 and 45°. These temperatures are in excellent agreement with the temperatures at which the present author has demonstrated that the structure of interfacial (vicinal) water undergoes some type of change, most likely a higher-order phase transition. (Note also the results are essentially independent of the nature of the anion present).

In view of the significance of the results obtained by Wiggins, Hurtado and the present author have repeated such experiments on a similar type porous media (Davison gel, #950) using a temperature gradient device (Scientific Industries, Inc., Model 675). Because of the availability of the gradient incubator it was possible for us to make measurements at more closely spaced intervals than had been possible for Wiggins. The results obtained agree quantitatively with the results obtained by Wiggins. (These data are discussed in a recent volume.<sup>19</sup>)

### *Enzyme Kinetics*

The polythermostat is uniquely well suited to measurements of the effects of temperature on rates of reaction, especially enzyme reactions. This is particularly true for reactions which can be followed spectrophotometrically: the sample tube is transferred directly from the gradient device to

the spectrophotometer. As each reading can usually be made in a matter of a minute or so, nearly simultaneous measurements are possible, and variability due to need for different stock solutions (which may "age" from one day to another) is eliminated. Besides the use of screw-cap culture tubes (or L-shaped tubes) Thunberg tubes can be used in gradient devices with vertical sample wells. Using such techniques we have made a preliminary investigation of the effects of temperature on the alkaline phosphatase reaction.<sup>20</sup>

### Other Studies

Polythermostats have been used in the author's laboratory for measurements of the effects of temperature on: (a) solubility (and mutual solubility of partially immiscible liquids); (b) partition of organic solutes between aqueous phase and an immiscible, organic liquid; and (c) equilibrium constants.

## Biological Systems

### Germination Studies

Polythermostats have been used in the present author's laboratory for the study of effects of temperature on rates (and extent) of germination.

Figure 9 shows the percent germination of turnip seeds as a function of time (in hours) measured at 19.9°. Similar germination curves were obtained in experiments at each of the 30 available, different temperatures (wherever germination occurred). In Figure 9 the maximum germination rate ( $\alpha$ ) is defined as the maximum slope (indicated by the dotted line). This parameter is similar to the intrinsic germination rate, determined by the logistics equation generally used in population studies. Other measures of the rate of germination used are the reciprocal of the time to reach either 16% or 50% germination.

Figures 10 and 11 show the log germination rates, respectively, for the 16 and 50%

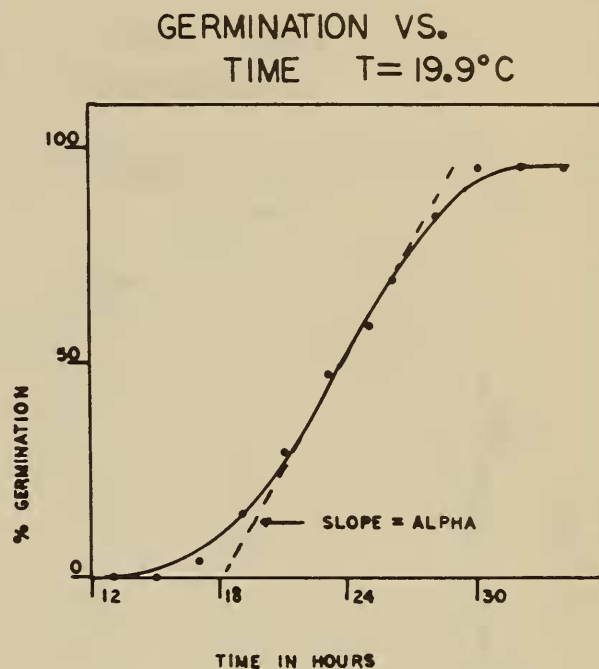


Fig. 9. Percent germination of turnip seeds as function of time. (Etzler and Drost-Hansen, 29)

germination plotted vs. reciprocal absolute temperature (in a standard type Arrhenius graph). As seen from Figures 10 and 11, it appears that changes in slope occur near 15 and 30°C. For a discussion of the kinetic interpretation of these data, see the paper by Etzler and Drost-Hansen.<sup>29</sup> Finally, Figure 12 shows the maximum rate of germination ( $\alpha$ , defined above) in an Arrhenius plot. A notable anomaly occurs near 30°C—again one of the temperatures at which vicinal water appears to undergo a marked change in structure.

In a separate series of experiments we have determined the effects of H<sub>2</sub>O replacement by D<sub>2</sub>O on the temperature dependence of the germination rate.<sup>21</sup> As the available polythermostats all have provisions for two samples at each of 30 different temperatures, one set of samples can be used as "control" (in this case, germination in pure H<sub>2</sub>O) while the other set of wells was used for measurement of germination in the presence of D<sub>2</sub>O. A total of three different D<sub>2</sub>O concentrations were investigated; 33, 67 and 98 mole-percent.

Figure 13 shows the amount of germination after 14 hours, for 67 mole-percent D<sub>2</sub>O. At low temperatures D<sub>2</sub>O notably in-

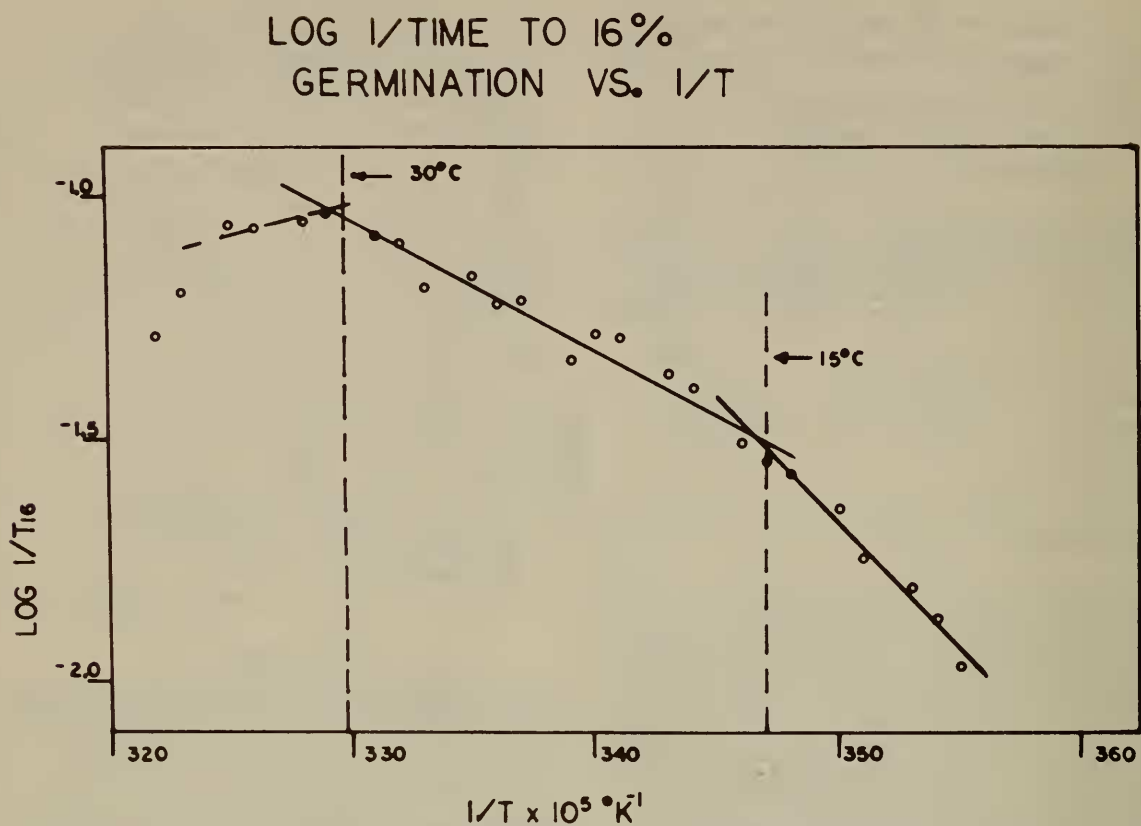


Fig. 10. Log germination rate for 16% germination of turnip seeds as function of reciprocal, absolute temperature. (Etzler and Drost-Hansen, 29)

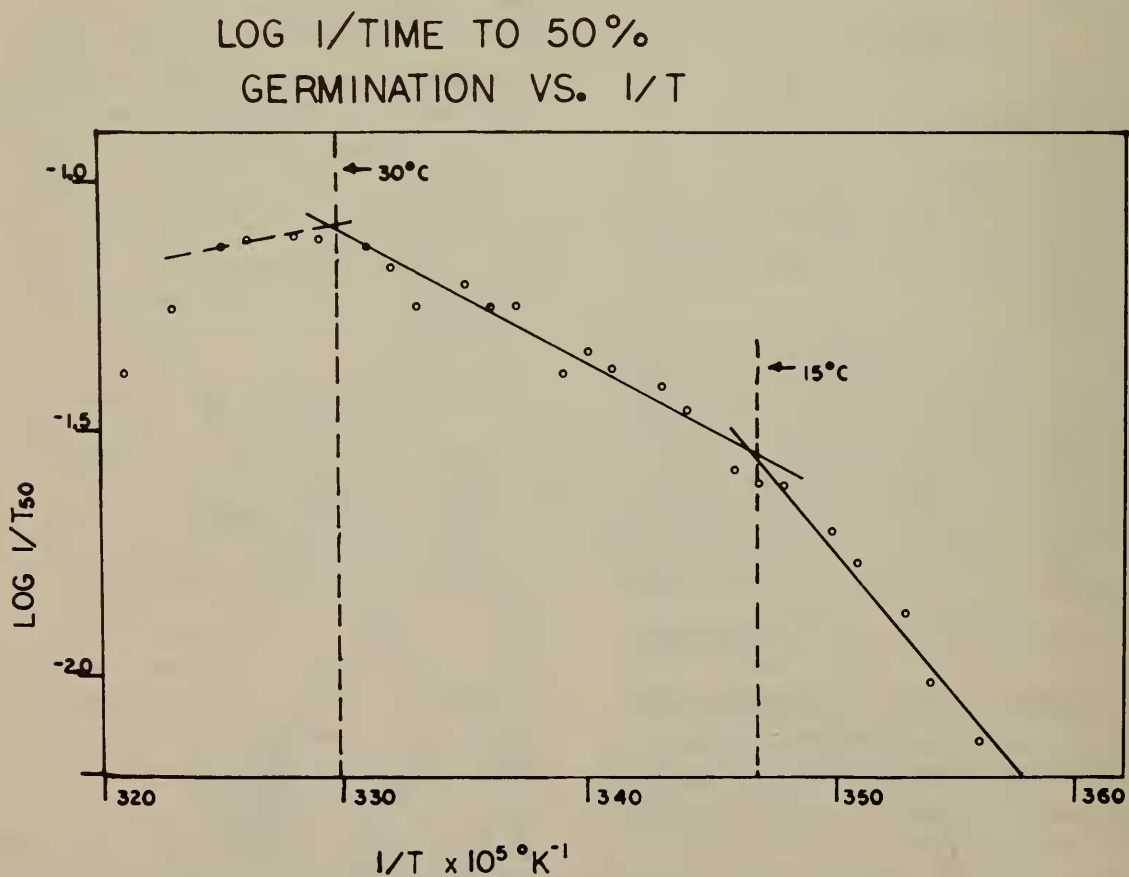


Fig. 11. Log germination rate for 50% germination of turnip seeds as function of reciprocal, absolute temperature. (Etzler and Drost-Hansen, 29)

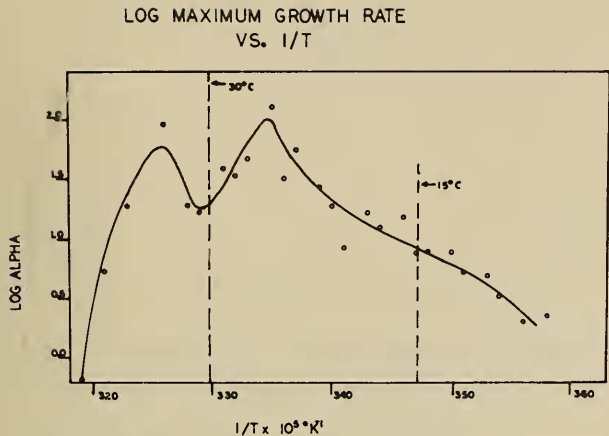


Fig. 12. Log maximum germination rate of turnip seeds as function of reciprocal, absolute temperature. (Etzler and Drost-Hansen, 29)

hibits the germination (reduces the rate of germination). However, at higher temperatures the effect of D<sub>2</sub>O replacement disappears; in fact, it appears that D<sub>2</sub>O may actually increase the rate of germination. We define an inhibition coefficient, I, as

$$I = 1$$

$$I = \frac{(\% \text{ germinated in H}_2\text{O-D}_2\text{O}) \text{ at time } t}{(\% \text{ germinated in H}_2\text{O}) \text{ at time } t}$$

For zero percent germination in an H<sub>2</sub>O-D<sub>2</sub>O mixture (when some germination has occurred in pure water), I is one. If no effect due to D<sub>2</sub>O is observed (i.e., identical amounts of germination), I is zero. If the percent of seeds germinated in D<sub>2</sub>O exceeds the percent germinated in H<sub>2</sub>O, I is negative and corresponds to a relative enhancement of germination. Figure 14 shows a graph of I as a function of temperature. Nearly complete inhibition is observed below ~26°C, but above this temperature the inhibition due to D<sub>2</sub>O decreases and at higher temperatures in a rather narrow range around 35 to 37°C it appears that germination is actually facilitated by D<sub>2</sub>O, however, this enhancement is not statistically significant.

### Algal Studies

Thorhaug, working in the author's laboratory a number of years ago, used a

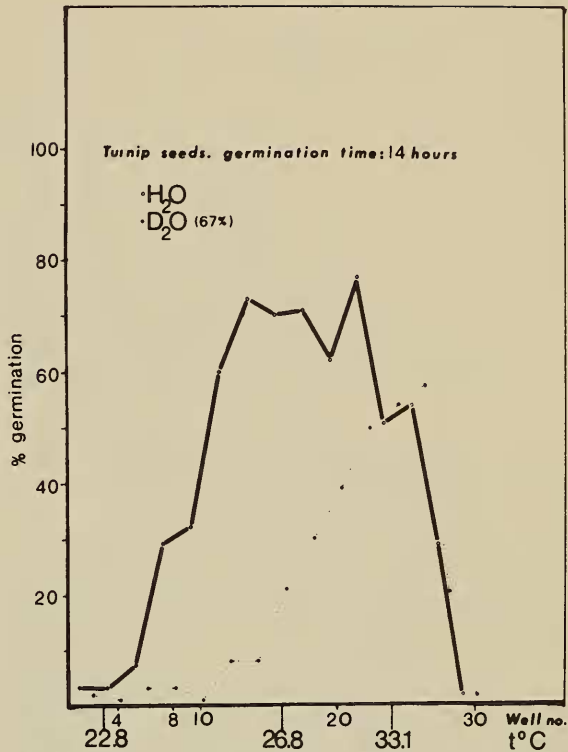


Fig. 13. Percent germination of turnip seeds in 67 mole-percent D<sub>2</sub>O as function of temperature after 14 hours. (Bee Drost-Hansen and W. Drost-Hansen, unpublished)

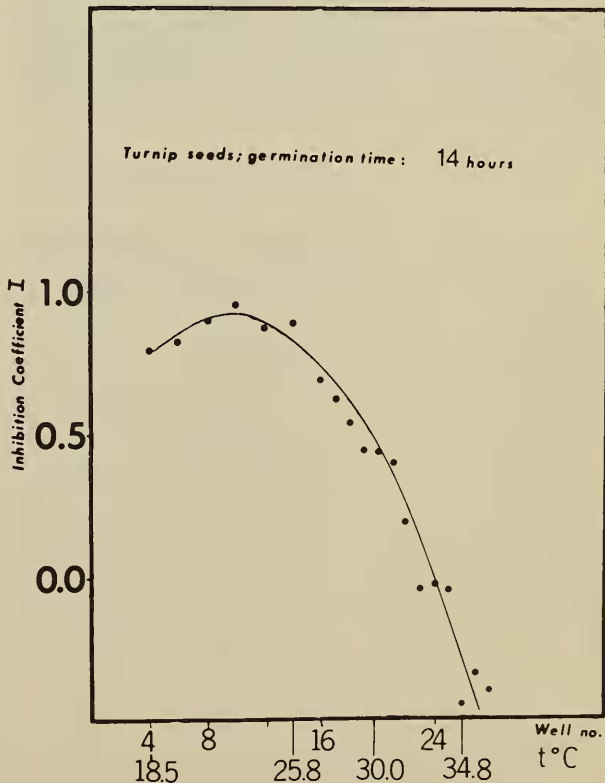


Fig. 14. Inhibition coefficient, I, (of germination) of turnip seeds as function of temperature. (Bee Drost-Hansen and W. Drost-Hansen, unpublished)

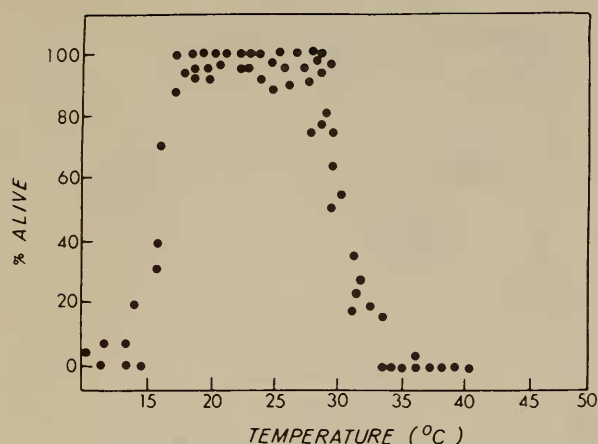


Fig. 15. Percent survival of *Valonia macrophysa* after 3 days exposure. (Thorhaug, 22)

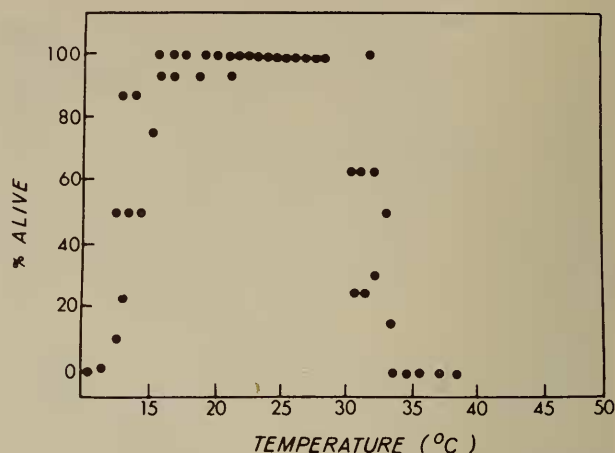


Fig. 17. Percent survival of *Valonia ventricosa* after 3 days of exposure. (Thorhaug, 22)

polythermostat to determine the effects of temperature on several species of algae, particularly *Valonia*.

In a series of experiments, Thorhaug<sup>22</sup> subjected three species of *Valonia* to temperatures ranging from 10°C to about 40°C. This study was undertaken in connection with our research on the possible existence of sharp upper thermal limits. The results of exposures of this alga to a wide range of temperatures, are shown in Figures 15, 16 and 17. The abruptness of the onset of irreversible plasmolysis is striking, both at the upper and lower ends of the temperature ranges (22; see also 23 and particularly 24).

#### Thermal Limits for Some Marine Organisms

Thorhaug<sup>22</sup> has reported data on the survival of a number of marine organisms

exposed to thermal stresses. A total of 27 different species and life stages were studied. Some of Thorhaug's results are shown in Figures 18, 19 and 20. In all cases shown, abrupt changes in survival occur near  $15 \pm 1^\circ\text{C}$  and near  $30 \pm 2^\circ\text{C}$ . The results are in excellent agreement with expectations based on the temperatures of changes in vicinal water structure.<sup>24,25,26,27</sup>

#### Microbial Growth Studies. Clostridium

The first use of the Oppenheimer/Drost-Hansen Polythermostat was to study the effects of temperature on the growth of a sulfate-reducing bacterium, probably a *clostridium*.<sup>16</sup> Notable growth optima were recorded at 12, 25 and 38°C with pronounced minima at 16, 31 and 45°C. These results were, however, obtained using an

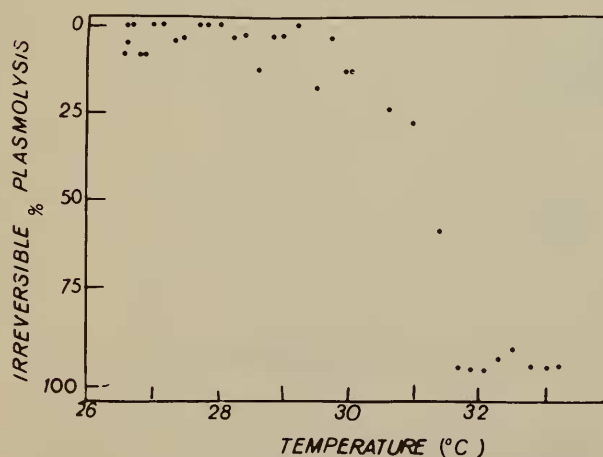


Fig. 16. Percent survival of *Valonia utricularis* after 3 days exposure. (Thorhaug, 22)

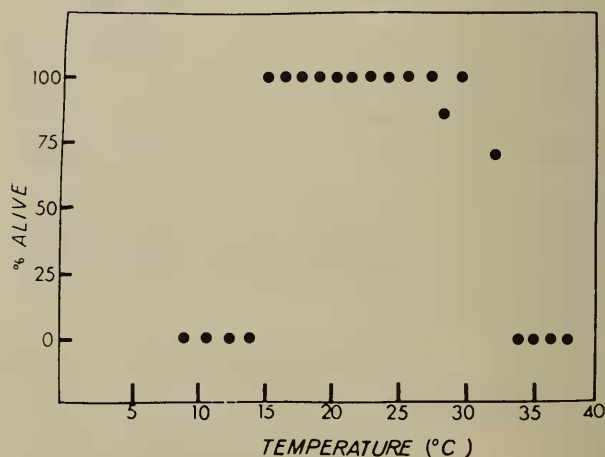


Fig. 18. Percent survival of *Penicillus capitatus* after 8 to 10 days exposure. (Thorhaug, 22)

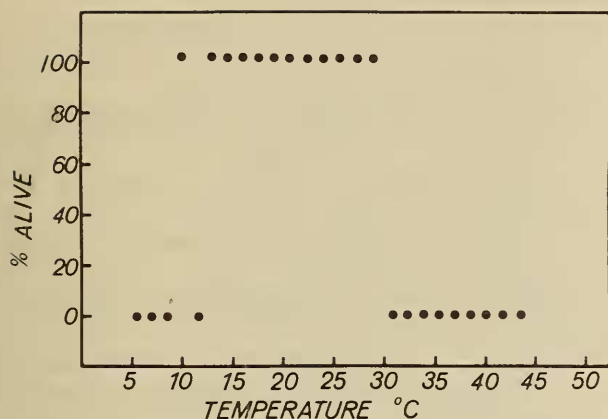


Fig. 19. Percent survival of *Menippe mercenaria* after 24 hours exposure. (Thorhaug, 22)

extremely simple approach (amounts of growth estimated only visually). For this reason, Schmidt and Drost-Hansen repeated the study more carefully and again observed growth optima near 24 and 40°C with a broad, somewhat asymmetric minimum near 31–33°C.<sup>28</sup> Schmidt and Drost-Hansen also measured the growth of a strain of *E. coli*, a strain of *Aerobacter* and *Serratia marcescens*. Again, multiple growth optima and minima were discovered especially where the organisms were grown on minimal media.

#### Multiple Growth Optima

In the original publication<sup>16</sup> Oppenheimer and Drost-Hansen proposed that the multiple growth optima might reveal the operation of two (or more) different metabolic pathways, for instance, above or below 31°C, somehow imposed by more or less abrupt changes in water structure. This

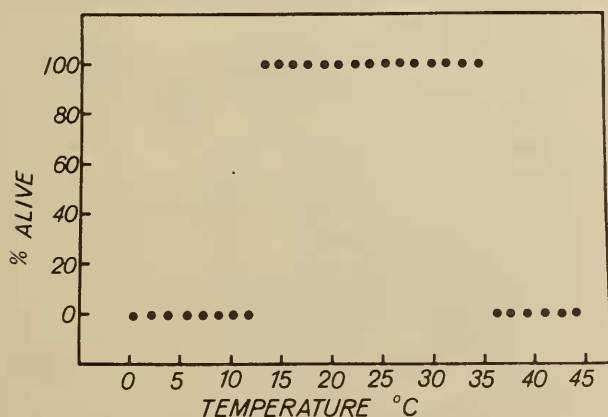


Fig. 20. Percent survival of *Periclimenes* sp. after 168 hours exposure. (Thorhaug, 22)

idea was also discussed by Drost-Hansen,<sup>26</sup> but little progress was made at the time; this was, in part, due to the mistaken notion that bulk water structure underwent structural changes near the observed transition temperatures. First by 1968 did it become obvious<sup>7</sup> that bulk water does not change, but the structure of vicinal water does. More recently the problem has been taken up again by Etzler and Drost-Hansen; the results of these studies have appeared recently.<sup>29,30</sup>

In passing, it should be noted that many other authors have reported multiple optima for growth and other biological processes. (Some of the pertinent papers are listed in 24). Only one other example of multiple growth optima will be mentioned briefly here. Etzler and Drost-Hansen<sup>30</sup> studied the growth of a green photosynthesizing thermophilic alga, *Cyanidium caldarium*. A typical growth curve is shown in Figure 21. No less than three optima have been observed (repeatedly) in these experiments. The optima occur near 28, 37 and 48°C, with the growth minima near 32 and 43°C—again in excellent agreement with the temperatures at which the thermal transitions are observed in vicinal water (near 14 to 16°; 29 to 32°; 44 to 46°C).

#### Summary and Discussion

Through measurements at closely spaced temperature intervals anomalous properties have been observed in a large number of aqueous interfacial systems, ranging from dispersed clays and highly porous silica gels to living (cellular) systems. A convenient device is described for studies at closely spaced temperatures. The anomalous effects observed are explained (in part) in terms of structural changes in vicinal (interfacial) water.

#### Acknowledgment

The author wishes to express his gratitude to the Environmental Protection

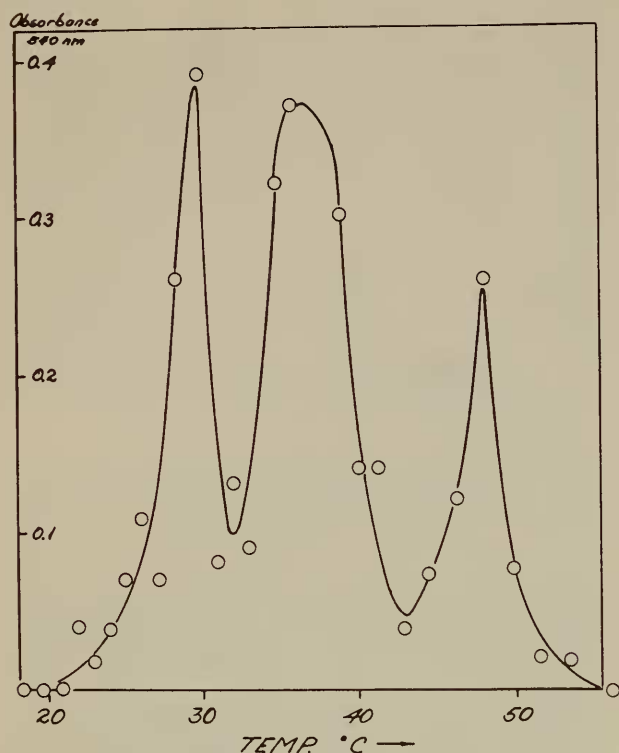


Fig. 21. Growth (as measured by optical density) of *Cyanidium caldarium* (a green, thermophilic alga) as function of temperature. (Etzler and Drost-Hansen, 30)

Agency (EPA) for its extended support of his research on aqueous systems. Thanks are also due to Scientific Industries, Inc. (Bohemia, New York) for donating a commercial model of its Temperature Gradient Incubator. Dr. James Clegg has greatly helped the author on innumerable occasions through advice, discussions and encouragement. Dr. Robert Cunnion conducted the enzyme experiments; Mrs. B. Drost-Hansen assisted with some of the germination studies, and Dr. Frank Etzler has contributed significantly through his work on seed germination and algal growth studies. My sincere thanks to all of these investigators.

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